The ***REAP***® enzyme supplement has two specific purposes:

1) Increase the rate and extent of protein and carbohydrate digestion in the intestines;
2) Shift the fermentation of fiber from the hindgut to enzymatic digestion in the small intestine; releasing useable nutrients. Poultry are poor fiber digesters.

REAP®

General Information :

REAP® is the dehydrated product of the fermentation of *Bacillus subtilis, Trichoderma longibrachiatum, Aspergillus niger* and *Aspergillus oryzae* with magnesium mica, calcium carbonate and dried distillers grains as carriers. ***REAP®*** contains carbohydrases {β-glucanase [1,3-(1,3;1-4)-β-glucan 3(4)-glucanohydrolase], and cellulase [Endo-1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase]} and protease as the main enzymes activities; with many additional enzymatic side activities [e.g. amylase (1,4-α- D-glucan glucanohydrolase) and xylanase (1,4- β-D-xylan xylanohydrolase]. ***REAP®*** has a very positive effect on improvement of nutrient availability and performance of poultry when it is supplemented to soy and grain-based diets at recommended supplementation rates.

Specification :

Major Enzyme activities

Protease	Min. 40 (Units/g)[1]
Cellulase	Min. 400 (Units/g)[2]
β-glucanase	Min. 600 (Units/g)[3]

[1] : One hemoglobin unit of protease produces, in 1 minute, a hydrolysate whose absorbance at 275 nm is equal to a solution containing 1.1 micrograms per mL of tyrosine in 0.006*N* hydrochloric acid at pH 4.7 and 40°C.

[2] : Once unit will produce a relative fluidity change of 1 in 5 minutes at pH 4.5 and 40°C

[3] : Unit is equal to 1 μmol total reducing sugars-glucose equivalent-released per 1 min. at 40℃ and pH 6.5.

Product Ingredients

- *Bacillus subtilis, Trichoderma longibrachiatum, Aspergillus niger* and *Aspergillus oryzae* fermentation extracts............Approximately 21% by weight.
- Magnesium mica, calcium carbonate and dried distillers grains............Approximately 79% by weight.

Main Nutrient Contents

Crude Protein	Min. 12%
Crude Fiber	Min. 20%
Crude ash	Max. 25%
Moisture	Max. 15%

General Effects (Efficacies):

- Enhances the nutritional value of soy and grain-based diet for monogastric animals.
- Reduces digesta viscosity of soy and grain-based diets; reduces non-starch polysaccharides in digesta; hydrolyzes proteins in the corn-soy diet.
- Improves the weight gains and feed conversion efficiencies of poultry.

Application Animals :

- Poultry (layers, broilers, turkeys, ducks).

Usage :

- Supplements it at optimum dosage to diets.
- Mix into complete mash feed or top-dress onto feed.

Dosage :

- Poultry : 0.5 – 1.0 lb/ton of complete feed.

Precaution :

- Work areas where exposure to forced dust occurs require good general ventilation.
- Avoid inhalation of dust.

Stability and Storage :

- 2 years from date of manufacturing.
- Store in dry and cool place.

Packaging methods:

- Net product weight is 50 lb.
- Packaging is four layers of 50 lb-test paper with a poly-layer, sewn bag.
- Product code and batch number are laser labeled onto each bag.

Manufacturer :

- Agri-King, Incorporated. Fulton, Illinois, USA.
- GMP, FEMAS, Safe Feed-Safe Food Certified manufacturing facility.

Summary of Pen Trials (2003-2007) Using Dietary Enzyme Blend ***REAP***® to Enhance Broiler Chicken Performance

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Enzymes serve as catalysts to speed up reactions, and in the case of ingredients in broiler chicken feeds as the substrates, to facilitate release of more nutrients during digestion for absorption and utilization in order to improve live and processed performance. This is particularly important in times when feed ingredients are high in cost, when less digestible alternative or byproduct ingredients rather than corn and soybean meal are used, or when the value of broiler meat is high. Endogenous enzymes are those released within the intestinal tract of the bird whereas exogenous enzymes are provided as a dietary supplement to broilers to provide additional enzyme activity.

This article summarizes broiler chicken feeding trials from 2003 to 2007 using a unique enzyme product (REAP®) developed by Agri-King, Inc., Fulton, IL and marketed by Prince Agri Products, Inc., Quincy, IL. Three forms of the product are commercially available for use in mash (REAP® 4M) or steam pelleted feeds (REAP® TS, meaning thermostable due to a coating), or for post-pelleting application (REAP® 50L). Both dry forms are granular, free-flowing products added at levels of 0.5 to 1.0 lb/ton (0.025 or 0.05%) of complete feed. The liquid enzyme product is added at 45 g/ton (equivalent to 0.025% dry forms).

These enzyme supplements contain fermentation extracts of *Aspergillus oryzae*, *Bacillus subtilis,* and *Trichoderma viride* with guaranteed minimum activities of β-glucanase (200,000 units/lb) and protease (5,000 HUT/lb) although several other unquantified enzymes from the fermentation process are also present. The main purpose for using these enzyme blend products is to make available more metabolizable energy from broiler diets. Metabolizable energy is comprised of energy from carbohydrates, proteins, and lipids (fats and oils).

Lower Brooding Temperature and Dietary Enzymes. A litter pen Trial 1 (Sims, 2003a) was conducted with day-old, straight-run Ross x Hubbard Hi-Y broiler chicks to evaluate the effects of the dietary enzyme product in corn-soy and animal-vegetable fat blend mash feeds on live performance at lower brooding temperatures (see Table 1). A total of 360 chicks were randomly distributed to 2 treatments (30 chicks/pen, 6 pens/ treatment), lower brooding temperature with or without dietary enzymes, at 0.67 ft^2/bird to 32 days of age. The corn-soy and animal-vegetable blend fat based starter diet used in Trial 1 was calculated to contain 22.0% crude protein, 1,400 kcal ME/lb, and 6.5% crude fat, but analyzed 25.8% crude protein, 36.8 to 37.8% starch, and 6.9 to 7.3% crude fat.

In Trial 1 (Table 2), body weight, feed consumption, feed conversion ratio, and mortality were significantly improved at lower brooding temperatures when the enzyme product was included in the diet, implying that some enhanced metabolizable energy utilization from the diet occurred. Interestingly, appetite appeared to be stimulated by the product possibly due to more rapid growth as a result of better nutrient digestibility.

Table 1. Cool stress brooding temperatures used in combination with or without dietary enzyme (REAP®, 0.05%) in Trials 1 and 2 (Sims, 2003, 2003a, and 2003b)

Age, days	Brooding temperatures (heat lamp), °F	
	Normal	Lower
Trial 1:		
0 to 4	-----	87
5 to 7	-----	85
8 to 14	-----	80
15 to 32	-----	75
Trial 2:		
0 to 2	94	87
3 to 5	92	85
6 to 8	90	82
9 to 11	88	80
12 to 14	85	78

In Trial 2a, 540 chicks were distributed to 3 treatments using 30 birds/pen and 6 pens/ treatment at an initial stocking density of 0.67 ft^2/chick. Treatments included negative control, bacitracin-md (55 ppm), or enzyme supplemented diets administered to chicks being brooded at lower than normal temperatures (see Table 1).

Results of Trial 2a are presented in Table 2. Compared to negative control group results, the bacitracin-md and enzyme fed groups had significantly greater body weight and feed consumption along with lower feed conversion ratio. These supplements were statistically equivalent for improving these 3 parameters. The respective uplifts in ME to the 1,400 kcal/lb starter diet were estimated at +303 and +414 kcal/lb feed under the cooler brooding conditions. The negative control group's caloric conversion (1.513 FCR x 1,400 kcal ME/lb feed = 2,118 kcal ME/lb body weight) was divided by each other treatment FCR, then 1,400 kcal ME/lb in starter feed was subtracted from it, to find apparent ME uplift due to each supplement. This assumes, for the purpose of calculation, that broilers in supplemented feed treatment groups needed the same equivalent (absolute) caloric intake as negative control birds per lb of body weight.

In Trial 2b, 1,080 chicks were divided into 6 treatments using 30 birds/pen and 6 pens/ treatment. The chicks were exposed to normal brooding temperatures (see Table 1) and given the same treatments as in Trial 2a, except that diets were in either mash or crumble form. The corn-soy and animal-vegetable blend fat based starter feed was calculated to contain 22.0% crude protein and 1,400 kcal ME/lb.

Results of Trial 2b are shown in Table 2. The negative control crumbled feed gave significantly increased body weight and feed consumption along with lower feed conversion ratio than negative control mash feed. The bacitracin-md and enzyme product were equally effective statistically at improving 14-day body weight and feed conversion ratio of broiler chickens fed either mash or crumbled negative control diets.

Table 2. Live performance of Ross x Hubbard Hi-Y broiler chicks in litter pens given lower brooding temperature regimens (Trial 1, see Table 1) or normal versus lower brooding temperatures (Trial 2a) and mash diets with bacitracin-md (0 or 55 ppm) or enzymes (REAP®, 0 or 0.05%) vs. similar crumbled diets (Trial 2b) (Sims. 2003, 2003a, 2003b)

Trial, Age, Brooding °F, Treatment	Body weight, lb	Feed consumption, lb/bird	Feed conversion ratio	Mortality, %
<u>Trial 1 (Lower °F brooding)</u>:				
Negative control, mash, 14 days	0.324^{b}	0.548^{b}	3.352^{a}	24.58^{a}
+ Enzymes, mash, 14 days	0.499^{a}	0.831^{a}	2.139^{b}	5.61^{b}
Negative control, mash, 32 days	1.127^{b}	2.825^{b}	4.403^{a}	62.30^{a}
+ Enzymes, mash, 32 days	2.197^{a}	4.881^{a}	2.437^{b}	18.68^{b}
<u>Trial 2a (Lower °F, 14 days)</u>:				
Negative control, mash	0.651^{b}	0.846^{b}	1.513^{a}	1.11
Bacitracin-md, mash	0.834^{a}	0.927^{a}	1.244^{b}	0
Enzymes, mash	0.875^{a}	0.915^{a}	1.168^{b}	0.56
<u>Trial 2b (Normal °F, 14 days)</u>:				
Negative control, mash	0.607^{c}	0.817^{b}	1.580^{a}	1.11
Bacitracin-md, mash	0.799^{a}	0.900^{a}	1.271^{c}	0
Enzymes, mash	0.803^{a}	0.907^{a}	1.270^{c}	1.11
Negative control, crumbles	0.706^{b}	0.902^{a}	1.467^{b}	1.11
Bacitracin-md, crumbles	0.836^{a}	0.971^{a}	1.298^{c}	0
Enzymes, crumbles	0.839^{a}	1.017^{a}	1.356^{c}	1.11

$^{a-b}$ Means within a column and grouping with a different letter superscript differ in Trial 1 by 2-Sample t-test or in Trial 2 by 1-Way ANOVA and LSD ($P \leq 0.05$).
$^{a-b}$ Means separated at $P \leq 0.05$ by 1-Way ANOVA for mash versus crumbles.

Trial 2b also involved a steam pelleting study to determine the enzymatic activity of the diets as mash or as crumbles manufactured at 3 steam conditioning temperatures (170, 195, or 210° F). The original non-coated enzyme product was used. No enzymatic activity was found in the negative control diet and 100% was found in the supplemented mash diet. Activities of β-glucanase and protease after exposure to the 3 conditioning temperatures (that is, post-pelleting) averaged 94.9, 95.3, and 78.2%, respectively, indicating good thermostability.

Based on 0-14 day feed conversion ratios in Trial 2b obtained under normal brooding temperatures and using mash starter feed (1,400 kcal ME/lb), the negative control birds required 2,212 kcal ME/lb body weight, respectively. The crumbled starter feed treatment groups needed only 2,054 kcal ME/lb body weight, respectively. With mash starter feed, the apparent uplift in ME with enzymes to the 1,400 kcal ME/lb diet appeared to be +342 kcal whereas with crumbled feed the uplift was estimated at +115 kcal/lb feed.

Dose Responses at Different Rates of Inclusion. Trial 3 involved supplementing broiler chick diets with 0, 0.015, 0.025, 0.0375, or 0.05% (0.25, 0.50, 0.75, or 1.00 lb/ton) of the enzyme product, plus enzymes at 0.05% and bacitracin-md at 55 ppm combined, to determine dose-associated responses. A total of 2,100 day-old, straight-run Ross x Hubbard Hi-Y broiler chicks were divided among 7 treatment groups (30 birds/pen, 10 pens/treatment). Initial stocking density was 0.67 ft^2/chick. Corn-soy and animal-vegetable fat based diets were used in a 3-phase feeding program with calculated nutrient values of 22, 20, and 18% crude protein and 1,400, 1,425, and 1,450 kcal ME/lb for starter, grower, and finisher diets (0-21, 21-42, and 42-49 days), respectively.

Table 3. Live performance and litter scores of broiler chicks to 49 days of age in litter pens as affected by dietary bacitracin-md (0 or 55 ppm) or enzyme product (0 to 0.05%) in Trial 3 (Sims, 2003c)

Dietary treatment	Body weight, lb	Feed consumption, lb/bird	Feed conversion ratio	Mortality, %	Litter score (0-4)[1]
Negative control	4.46[c]	7.31[b]	2.011	5.67	2.40[a]
+ Bacitracin-md (55 ppm)	4.77[b]	7.89[a]	1.983	4.33	1.50[b]
+ Enzymes (0.0125%)	4.94[ab]	8.00[a]	1.913	3.32	1.60[b]
+ Enzymes (0.025%)	4.90[ab]	7.83[a]	1.899	2.33	1.70[ab]
+ Enzymes (0.0375%)	5.09[a]	8.27[a]	1.945	2.00	1.00[b]
+ Enzymes (0.05%)	4.87[ab]	7.91[a]	1.962	3.00	1.40[b]
+ Bacitracin (55 ppm) & Enzymes (0.05%)	5.10[a]	8.23[a]	1.931	2.00	1.00[b]
P value	0.001	0.004	0.162	0.239	0.004

[a – b]Means within a column and having a different letter superscript differ by 1-Way ANOVA and LSD at $P \leq 0.05$.

[1]Litter scores, 0 to 4, best to worst: 0 = normal, mostly dry; 1 = slightly wet or discolored litter; 2 = moderately wet or discolored litter; 3 = moderately wet and discolored litter; 4 = severely wet litter.

Table 4. Fresh excreta assays for chemical composition and microbial profiles at different ages as affected by dietary bacitracin-md (55 ppm) or enzyme product (0.05%) in Trial 3 (Sims, 2003c)

Parameters	Age, days	Dietary treatment		
		Negative control	+ Bacitracin-md	+ Enzymes
Composition (100% DM):[1]				
Crude protein, %	14	37.01	44.49	39.50
	21	20.49	19.62	21.29
	42	31.42	33.60	28.13
	49	30.41	25.66	25.89
	Avg (*P*=0.126)	29.83	30.84	28.70
Starch, %	14	2.84	0.65	0.70
	21	1.99	1.08	0.88
	42	2.37	1.26	0.93
	49	3.15	4.53	1.87
	Avg (*P*=0.007)	2.59[a]	1.88[ab]	1.10[b]
Microbial profiles:				
Clostridia, cfu/g	21	5.50×10^7	1.25×10^7	2.75×10^7
	42	6.00×10^6	2.40×10^7	1.30×10^7
	49	3.48×10^6	9.63×10^5	1.93×10^6
	Avg (*P*=0.552)	2.15×10^7	1.25×10^7	1.41×10^7
Lactic acid bacteria, cfu/g	21	2.98×10^8	2.96×10^8	3.10×10^8
	42	4.40×10^9	3.00×10^9	3.99×10^9
	49	3.20×10^9	2.41×10^9	2.31×10^9
	Avg (*P*=0.213)	2.63×10^9	1.90×10^9	2.20×10^9
Salmonella, log10 cfu/g	21	3.40×10^7	2.00×10^6	3.00×10^6
	42	4.00×10^7	2.00×10^8	4.10×10^7
	49	3.48×10^8	9.63×10^6	4.81×10^5
	Avg (*P*=0.375)	1.41×10^8	7.05×10^7	1.48×10^7

[a – b]Means within a row and having a different letter superscript differ by repetitive Paired t-test at *P* value indicated.
[1]100% dry matter basis.

In Trial 3 (Table 3), body weight at 49 days was significantly increased over negative control by all enzyme, bacitracin-md, and combined treatments. Similarly feed consumption was increased and litter scores improved by all other treatments compared to negative control. Fresh excreta assay results shown in Table 4 revealed that starch content on a 100% dry matter basis was significantly lower in the enzymes-fed group compared to the negative control group and statistically equivalent to the bacitracin-md group's starch content.

The weighted average caloric conversion for the negative control group in Trial 3 (Table 3) was calculated as 2,645 kcal ME/lb body weight for 0-49 days (3 phases). The feed consumption pattern was 30.77% starter (1,919.4 kcal/lb body weight), 57.32% grower (3,327.4 kcal/lb body weight), and 11.91% finisher (1,236.9 kcal/lb body weight) in the negative control group. The average consumption pattern for the enzyme supplemented diets was 28.02% starter (0-21 days), 60.03% grower (21-42 days), and 11.95% finisher (42-49 days). Using feed conversion ratios from 0-21 days and feed/gain ratios from 21-42 and 42-49 days, the apparent uplifts in ME due to various levels of enzyme addition were calculated as follows during starter, grower, and finisher phases: 0.0125%, +27, +119, -117; 0.025%, +1, +179, -56; 0.0375%, +23, +236, -321; and 0.05%, +5, +87, -41. During the finisher period, the feed/gain was unusual in that in 6 of 7 treatments more weight gain occurred than feed consumed, but the reason in unknown. Using the body weight and feed consumption data by period for each of the enzyme supplemented treatments, estimated apparent uplifts in ME overall from 0-49 days were: 0.0125%, +64; 0.025%, +101; 0.0375%, +111; and 0.05%, +48 kcal/lb feed.

Regular, Coated, and Liquid Enzyme Products Compared. In Trial 4 (Sims, 2007), 960 day-old, straight-run Cobb x Ross broiler chicks were assigned to 4 treatment groups (8 litter pens of 32 chicks each per treatment). Initial stocking density was $0.67 ft^2$/chick. Treatments included either a single negative control diet with corn-soy and 10% DDGS (calculated 20% crude protein, 1,425 kcal ME/lb, 7.22% crude fat) or the same basal diet supplemented with either regular, coated, or liquid (spray) enzyme product ((REAP®). Regular and coated enzyme supplements were added at the mixer, and the liquid enzyme product (50 g/ton) was sprayed on post-pelleting. The liquid supplement provided enzyme activities equal to those obtained from a level of 0.05% regular or coated enzyme product.

The negative control diet was pelleted at 192 to 200° F, and the diets for the other 3 treatments were pelleted at 200 to 202° F. The activities of β-glucanase and protease in regular enzyme product diets post-pelleting averaged 39.1% of those in the coated enzyme product diets. The diet with the liquid enzyme supplement applied post-pelleting had enzyme activities averaging 87.7% of those in the coated enzyme product diets. Results to 46 days of age are shown in Table 5.

In Trial 4 (Table 5), all three delivery forms of the enzymes (regular, coated, and liquid) significantly increased body weight and improved mortality-adjusted feed conversion ratio compared to negative control. Mortalities were probably higher in enzyme treatments due to stresses of more rapid growth and high stocking density in small pens.

Because a single 20% protein and 1,425 kcal ME/lb diet was used in Trial 4 from 0-46 days, it is simple to estimate the negative control treatment group's caloric conversion by multiplying adjusted feed conversion ratio (2.020) by 1,425 kcal/lb. The result was 2,879 kcal ME/lb body weight. Using the negative control's caloric conversion, adjusted feed conversion ratios for enzyme treatments, and the calculated ME content of the negative control feed, it was possible to calculate apparent uplifts in ME for the enzyme supplemented treatments as follows: regular enzymes, +41; coated enzymes, +62; and liquid enzymes post-pelleting, +101 kcal/lb feed.

Table 5. Live performance of Cobb x Ross broiler chickens at high stocking density (0.67 ft^2/chick initially) in litter pens as affected by treatments including regular, coated, or post-pelleting liquid spray-on enzyme product to 46 days of age in Trial 4 (Sims, 2007)

	Body weight, lb	Feed conversion ratio	Adj. Feed Conv. Ratio[1]	Mortality, %[2]
Negative control	6.133[b]	2.046	2.020[z]	3.33[b]
+ Regular enzymes	6.377[ab]	2.058	1.963[ab]	7.50[a]
+ Coated enzymes	6.506[a]	2.006	1.936[b]	7.50[a]
+ Liquid enzymes (spray)	6.351[a]	1.947	1.886[b]	7.08[ab]

[a–b]Means in a column and with a different letter superscript differ ($P \leq 0.05$).
[1]Mortality-adjusted feed conversion ratio (weights recorded for death losses).
[2]Higher mortalities in enzyme treatments likely due to stresses of rapid growth and high stocking density (30 birds per 4' x 5' pen). Mortality percentages to 46 days with cardiac disorder losses removed were 2.92, 2.92, 3.75, and 3.75, respectively ($P > 0.05$).

Various Dietary Fat Levels to Quantify Enzyme Benefits. A 49-day broiler feeding trial (Trial 5) in litter pens was conducted with corn-soy, DDGS (5%), and poultry byproduct meal (3%) based diets containing either 1.00, 2.71, 4.42, or 6.13% added poultry fat in starter or 1.00, 2.25, 3.75, or 5.00% added poultry fat in grower and finisher feeds. The 1.00% poultry fat diets were supplemented with either 0, 0.025, or 0.05% coated enzyme product (REAP® TS). All starter, grower, and finisher feeds contained monensin (90 g/ton) and bacitracin-md (55 ppm) and were fed as crumbles or pellets. A total of 2,400 day-old male Cobb x Cobb broiler chicks were assigned to the 6 treatment groups (8 litter pens of 50 chicks per treatment). Initial stocking density was 0.93 ft^2/chick. The trial was conducted in winter. Results are presented in Table 6.

In Trial 5 (Table 6), addition of 0.025 coated enzyme product significantly improved 21- and 49-day body weight compared to 1.00% poultry fat basal diets. At 49 days, the basal diets with 0.05% coated enzyme product significantly increased body weight and decreased feed conversion ratio compared to 1.00% poultry fat basal diets. Mortality by treatment was variable at both ages. Mortality-adjusted feed conversion ratio from 0 to 49 days was found to be = 1.9392-0.0012(poultry fat %) with $r^2 = 0.615$ at $P < 0.01$. The 0.025% level of enzyme product was found to be equivalent to 26.83 lb poultry fat/ton (1.34%), and the 0.25% level of enzyme product was equivalent to 41.83 lb poultry fat/ton (2.09%) in the 1.00% poultry fat basal diets.

Table 6. Live performance of Cobb x Cobb male broiler chickens in litter pens to 49 days of age as affected by level of added poultry fat (1.00 to 6.13%) and level of coated enzyme product (0, 0.025, or 0.05%) in 3-phase feeds in Trial 5 (Mathis, 2007)

Dietary treatment	21 days of age			49 days of age		
	Body weight, lb	Adj. feed conversion ratio[1]	Mortality, %	Body weight, lb	Adj. feed conversion ratio[1]	Mortality, %
1.00% Poult. Fat	1.40[b]	1.624[a]	2.80[ab]	6.33[d]	1.923[a]	4.20
+ 0.025% Enzy.	1.55[a]	1.583[a]	2.20[ab]	6.74[a]	1.907[a]	6.20
+ 0.05% Enzy.	1.50[b]	1.571[a]	4.60[a]	6.58[bc]	1.889[b]	7.40
2.25-2.71% PFat	1.50[b]	1.566[a]	1.20[b]	6.61[abc]	1.868[bc]	4.80
3.75-4.42% PFat	1.50[b]	1.555[a]	2.40[ab]	6.60[bc]	1.848[c]	7.00
5.00-6.13% PFat	1.54[ab]	1.501[b]	2.60[ab]	6.71[ab]	1.819[d]	5.80

[a–d]Means within a column and with a different letter superscript differ ($P \leq 0.05$).
[1]Mortality-adjusted feed conversion ratio (death losses weighed).

Because 0-21 day feed consumption was clearly attributable to starter feed (estimated to have about 1,330 kcal ME/lb although not stated in the final report), the caloric conversion for the 1.00% poultry fat basal diet was calculated (adjusted feed conversion ratio x 1,330) to be 2,160 kcal/lb body weight. Based on the caloric conversion for the negative control diet and adjusted feed conversion ratios for the enzyme supplemented diets, the apparent uplifts in ME were estimated to be +34 kcal ME/lb feed with the 0.025% enzyme level and +45 kcal ME/lb feed for the 0.05% enzyme inclusion rate.

Low Protein Grower-Finisher Feeds With Enzyme Product. A total of 960 day-old, straight-run Cobb x Cobb High Yield broiler chicks were allocated to 3 treatment groups (8 litter pens of 40 chicks per treatment). The negative control starter diet was calculated to have 23% protein, and 0 or 0.05% enzyme product was added to it (0-18 days). The negative control and supplemented starter diets assayed 21.33 and 22.65% protein, respectively. During the grower-finisher period (18-42 days), the negative control basal diet had 16% protein, and to this was added 0 or 0.05% enzyme product. These diets assayed 15.96 and 16.46% protein, respectively. A 24% protein grower-finisher regimen was used to try to achieve 1-2% more carcass yield and 3-4% more breast yield compared to the 16% protein grower-finisher diet. It assayed 23.46% protein. Feed consumption was expected to be about 12% starter and 88% grower-finisher. All diets had virginiamycin (11 ppm) and monensin (99 ppm). The trial was conducted from February 12-March 27, 2007. Results are given in Table 7.

In Trial 6 (Table 7), addition of 0.05% level of enzyme product to 16% protein grower-finisher feed significantly improved body weight and adjusted feed conversion ratio compared to the negative control. Improvements were not as great as with the high protein regimen. Breast meat % and abdominal fat pad % of live weight were greatly improved with the high protein grower-finisher regimen compared to the lower protein regimen with or without enzymes.

Table 7. Effects of dietary grower-finisher feed (21-42 day) protein levels with or without enzyme product (REAP®) on live performance of Cobb x Cobb High Yield broiler chicks to 42 days of age in litter pens and on day 43 carcass characteristics in Trial 6 (Pesti, 2007)

Dietary treatment (protein by feed phase: starter, grower-finisher)	Body weight, lb	Adj. feed conversion ratio	Mortality, %	Chilled carcass, % live weight	Breast meat, % live weight	Abdominal fat pad, % live weight
23% S, 16% GF	5.45[b]	1.865[a]	3.38	74.70	22.46[b]	2.48[b]
23% S, 16% GF + 0.025% Enz.	5.58[b]	1.763[b]	2.79	74.82	22.92[b]	2.44[b]
23% S, 24% GF	6.13[a]	1.593[c]	4.50	75.68	27.30[a]	1.26[a]

[a–b]Means within a column and with a different letter superscript differ ($P \leq 0.05$).

The 16% grower-finisher fed negative control birds had 21-42 day mortality adjusted feed conversion ratio of 2.077 using a 1,452 kcal ME/lb diet, thus giving a caloric conversion of 3,016 kcal ME/lb body weight. Using this information and the adjusted feed conversion ration of the 0.05% enzymes treatment (1.948), the apparent uplift in ME was calculated to be +96 kcal/lb feed.

Body weight gain during the 21-42 day grower-finisher period was 0.311 kg more for the 24% protein diet group compared to the 16% protein diet group (negative control), or in other words, as a result of having an additional 8% dietary protein (0.0389 kg body weight/1% extra protein). Therefore, the enzyme product uplift of 0.055 kg in body weight above the negative control, was equivalent to +1.41% protein. Chilled carcass weight was improved by 0.284 kg with 24% compared to 16% protein grower-finisher diets (0.0355 kg/1% protein). Therefore, the enzyme product uplift of 0.059 kg chilled carcass was equivalent to 1.66% protein. Breast meat weight was improved by 0.168 kg with 24% compared to 16% grower-finisher diets (0.021 kg/1% protein). Therefore, the enzyme product uplift of 0.0286 kg breast meat using the 16% protein diet was equivalent to +1.05% protein. On average across these 3 parameters (body weight, chilled carcass, and breast meat), the enzyme product was equivalent to +1.37% extra dietary crude protein (that is, 16% protein + 0.05% enzymes was equivalent to 17.37% protein). For feed formulation purposes, the apparent extra 1.37% crude protein from corn and soybean meal would contribute the following approximate amino acid levels to the diet due to improved protein utilization: Lys 0.075, Met 0.036, Cys 0.024, Trp 0.017, Thr 0.068, Gly 0.061, Arg 0.095, Ile 0.071, Leu 0.127, Phe 0.069, and His 0.036%.

Enzymes in Grower-Finisher Feeds During July-September. Trial 7 involved 1,920 day-old male Cobb x Cobb chicks allocated to 6 treatment groups (8 litter pens of 40 chicks each per treatment). All birds received the same 23% protein and 1,344 kcal ME/lb starter diet from 0-21 days of age. From 21-49 days, 16 or 20% protein and 1,462 kcal ME/lb grower-finisher diets with 0, 0.025, or 0.05% enzyme product (REAP® TS) comprised 6 treatments. The low and high protein grower-finisher feeds contained 3.00 and 4.92-4.96% poultry fat, respectively. All of the corn-soy and poultry byproduct (2%) diets were given as crumbles or pellets and contained bacitracin-md (55 ppm) and monensin (99 ppm). Initial stocking density was 0.91 ft^2/chick. Results are presented in Table 8 (Mathis, 2007a).

Table 8. Effects of low or high protein broiler grower-finisher diets with or without enzyme product (REAP® TS) in pelleted form from 21-49 days of age in litter pens on 21-49 and 0-49 day live performance during July-September in Trial 7 (Mathis, 2007a)[1]

Dietary treatment (% protein in grower-finisher)	21-49 days of age			0-49 days of age		
	Weight gain, lb	Adj. feed conversion ratio[2]	Mortality, %	Body weight, lb	Adj. feed conversion ratio[2]	Mortality, %
16% Protein GF	3.993[d]	2.19	6.25	5.60[c]	1.98	7.81
+ 0.025% Enzy.	4.101[c]	2.10	3.13	5.67[c]	1.92	3.75
+ 0.05% Enzy.	4.048[cd]	2.14	3.44	5.67[c]	1.95	4.38
20% Protein GF	4.334[b]	2.03	3.75	5.97[b]	1.87	4.69
+ 0.025% Enzy.	4.429[b]	1.97	4.38	6.02[b]	1.84	5.31
+ 0.05% Enzy.	4.588[a]	1.95	3.75	6.26[a]	1.81	3.75

[a–b]Means within a column and with a different letter superscript differ ($P \leq 0.05$).
[1]All broilers chicks received the same starter feed from 0-21 days of age.
[2]Mortality-adjusted feed conversion ratio (death losses weighed).

In Trial 7 (Table 8), Cobb x Cobb male broiler chicks had significantly greater body weight gain from 21-49 days when 16% protein grower-finisher was supplemented with 0.025% of the enzyme product or when 20% protein grower-finisher had 0.05% of the enzyme product added compared to respective unsupplemented controls. The 49-day body weight was significantly improved when the 20% protein grower-finisher had been supplemented with 0.05% of the enzyme product during the 21-49 day period.

Caloric conversion from 21-49 days in Trial 7 for the 16% protein grower-finisher basal diet (1,462 kcal ME/lb) was 3,203 kcal ME/lb body weight and for the 20% protein grower-finisher basal diet (1,462 kcal ME/lb) was 2,968 kcal ME/lb body weight. The apparent uplift in ME in the 16% protein + 0.025% enzymes diet was +63 and in the 16% protein + 0.05% enzymes diet was +35 kcal ME/lb feed. The apparent uplift in ME in the 20% protein + 0.025% enzymes diet was +45 kcal and in the 20% protein + 0.05% enzymes diet was +60 kcal ME/lb feed.

Meta-Analysis of Enzyme Blend Effects on Broiler Live Performance. Table 9 contains results of a statistical meta-analysis of data from 6 trials comparing each enzyme treatment mean with the corresponding negative control treatment mean by paired t-test (16 paired comparisons). Overall, significant improvements in body weight and feed conversion ratio were obtained from supplementation of diets with the enzyme product (REAP®). Mortality was not significantly affected by enzyme supplementation.

Table 9. Meta-analysis summary of effects of the dietary enzyme product (REAP®) on body weight (BW), feed conversion ratio (FCR or Adj. FCR), and mortality of broiler chickens under normal or stressful conditions in litter pen trials from 2003 to 2007[1].

Trial	Treatment	Birds/ treatment (rep. pens)	Age, days	BW, lb	FCR or Adj. FCR[2]	Mortality, %
2a	Lower °F, negative control	180 (6)	14	0.651	1.513	1.11
2a	Lower °F, enzymes (0.05%)	180 (6)	14	0.874	1.168	0.56
2b	Normal °F, negative control	180 (6)	14	0.607	1.580	1.11
2b	Normal °F, enzymes (0.05%)[3]	180 (6)	14	0.803	1.270	1.11
2b	Normal °F, negative control	180 (6)	14	0.706	1.467	1.11
2b	Normal °F, enzymes (0.05%)	180 (6)	14	0.839	1.356	1.11
3	Negative control	300 (10)	49	4.46	2.011	5.67
3	+ Enzymes (0.0125%)	300 (10)	49	4.94	1.913	3.32
3	+ Enzymes (0.025%)	300 (10)	49	4.90	1.899	2.33
3	+ Enzymes (0.0375%)	300 (10)	49	5.09	1.945	2.00
3	+ Enzymes (0.05%)	300 (10)	49	4.87	1.962	3.00
4	Negative control	240 (8)	46	6.133	2.020	3.33
4	+ Regular enzymes (0.05%)	240 (8)	46	6.377	1.963	7.50
4	+ Coated enzymes (0.05%)	240 (8)	46	6.506	1.936	7.50
4	+ Liquid enzymes (0.05% eq.)	240 (8)	46	6.351	1.886	7.08
5	1.00% Poultry fat	400 (8)	49	6.33	1.923	4.20
5	+ Enzymes (0.025%)	400 (8)	49	6.74	1.907	6.20
5	+ Enzymes (0.05%)	400 (8)	49	6.58	1.889	7.40
6	23% S, 16% GF Protein	160 (4)	42	5.45	1.865	3.38
6	+ Enzymes (0.05%)	160 (4)	42	5.58	1.763	2.79
7	16% Gro-Fin Protein	320 (8)	49	5.60	1.98	7.81
7	+ Enzymes (0.025%)	320 (8)	49	5.67	1.92	3.75
7	+ Enzymes (0.05%)	320 (8)	49	5.67	1.95	4.38
7	20% Gro-Fin Protein	320 (8)	49	5.97	1.87	4.69
7	+ Enzymes (0.025%	320 (8)	49	6.02	1.84	5.31
7	+ Enzymes (0.05%)	320 (8)	49	6.26	1.81	3.75
Negative control		277	41.9	4.765[b]	1.887[a]	4.281
+ Enzymes (0.0412% avg)		277	41.9	4.945[a]	1.787[b]	4.119
Paired t-test, *P* value (n = 17)[4]				0.001	< 0.001	0.813
Actual change vs. neg. control				+0.180	-0.100	0.162
Relative change vs. neg. control, %				+3.78	-5.30	-3.78

[a–b]Overall means with different letter superscripts differ ($P \leq 0.05$).
[1]Day-old chicks in Trials 2-4 were straight-run and in Trials 5-7 were males.
[2]Adjusted FCR was used as first choice, but where it was not available FCR was used.
[3]REAP® at 0.05% supplied β-glucanase (200,000 units) and protease (5,000 HUT) activities per ton of feed.

[4]Each result from an enzyme supplemented treatment was compared with corresponding negative control result (17 paired comparisons from 6 trials); Trial 1 data omitted as outliers.

Table 10. Effects of bacitracin-md (55 ppm, positive control) versus enzyme product (REAP®, 0.0125-0.05%) supplemented diets for broiler chickens on body weight (BW), feed conversion ratio (FCR), and mortality in Trials 2 and 3 in 2003 (Sims, 2003a, 2003b, 2003c)

Trial	Treatment	Birds/ treatment (rep. pens)	Age, days	BW, lb	FCR or Adj. FCR[2]	Mortality, %
2a	Lower °F, bacitracin-md	180 (6)	14	0.834	1.244	0
2a	Lower °F, enzymes (0.05%)	180 (6)	14	0.874	1.168	0.56
2b	Normal °F, bacitracin-md	180 (6)	14	0.799	1.271	0
2b	Normal °F, enzymes (0.05%)[3]	180 (6)	14	0.803	1.270	1.11
2b	Normal °F, bacitracin-md	180 (6)	14	0.836	1.298	0
2b	Normal °F, enzymes (0.05%)	180 (6)	14	0.839	1.356	1.11
3	Bacitracin-md (55 ppm)	300 (10)	49	4.77	1.983	4.33
3	+ Enzymes (0.0125%)	300 (10)	49	4.94	1.913	3.32
3	+ Enzymes (0.025%)	300 (10)	49	4.90	1.899	2.33
3	+ Enzymes (0.0375%)	300 (10)	49	5.09	1.945	2.00
3	+ Enzymes (0.05%)	300 (10)	49	4.87	1.962	3.00
Bacitracin-md (55 ppm)		249	34.0	3.078[b]	1.678	2.474
Enzymes (0.0393% avg)		249	34.0	3.188[a]	1.645	1.919
Paired t-test, P value (n = 7)[2]				0.042	0.133	0.354
Actual change vs. bacitracin-md				+0.110	-0.033	-0.555
Relative change vs. bacitracin-md, %				+3.57	-1.97	-22.4

[a–b]Overall means with different letter superscripts differ ($P \leq 0.05$).

[1]REAP® at 0.05% supplied β-glucanase (200,000 units) and protease (5,000 HUT) activities per ton of feed in the mixer.

[3]Each result from an enzyme supplemented treatment was compared with corresponding bacitracin-md positive control result (7 paired comparisons from 2 trials).

Limited results from a meta-analysis comparing bacitracin-md (55 ppm) supplemented broiler diets versus enzyme product (0.0125-0.05%) supplemented diets are given in Table 10 (7 paired comparisons). Body weight was significantly increased by dietary enzymes compared to bacitracin-md.

Summary of Apparent Uplift in ME of Broiler Diets by the Enzyme Blend. Table 11 lists the estimated caloric improvements in broiler diets in Trials 2-7 in this report. At an average inclusion rate of 0.040% of the diet, the enzyme blend product provided an additional +68.1 kcal ME/lb feed to broiler chickens.

Table 11. Listing of metabolizable energy apparent uplifts with enzyme blend diets compared to negative control diets in broiler pen Trials 2 – 7 (2003 – 2007)[1]

Trial	Treatment	Age, days	Feed form	Enzyme product, %	Apparent uplift in ME, kcal/lb
2b	Normal °F brooding	0-14	Crumbles	0.05	+115
3	Basal starter + enzymes	0-49	Mash	0.0125	+64
3	Basal starter + enzymes	0-49	Mash	0.025	+101
3	Basal starter + enzymes	0-49	Mash	0.0375	+111
3	Basal starter + enzymes	0-49	Mash	0.05	+48
4	Regular enzymes	0-46	Crum./Pell.	0.05	+41
4	Coated enzymes	0-46	Crum./Pell.	0.05	+62
4	Liquid enzymes (spray on)	0-46	Crum./Pell.	0.05 (eq.)	+101
5	Basal starter + enzymes	0-21	Crumbles	0.025	+34
5	Basal starter + enzymes	0-21	Crumbles	0.05	+45
6	16% protein GF + enzymes	21-42	Pellets	0.05	+96
7	16% protein GF + enzymes	21-49	Pellets	0.025	+63
7	16% protein GF + enzymes	21-49	Pellets	0.05	+35
7	20% protein GF + enzymes	21-49	Pellets	0.025	+45
7	20% protein GF + enzymes	21-49.	Pellets	0.05	+60
Overall averages (n = 15):				0.040	+68.1

[1]Results from Trial 1 omitted as outliers. Trial 2a result using lower °F brooding and mash feed with 0.05% enzymes was +413 kcal ME/lb feed, not used (outlier). Trial 2b result using normal °F brooding and mash feed with 0.05% enzymes was +342, not used (outlier).

A linear regression was conducted using level of enzyme supplementation and ME uplift response based on data in Table 11 from the 6 trials. The regression was nonsignificant (ME uplift, kcal/lb feed = 62.6423 + 135.610(Enzyme product, %); r2 = 0.004; P = 0.821) indicating variability in the dose-responses. Variability was perhaps due to differences in the experimental diets such as enzyme product, fat source, fat level, animal protein ingredients, physical form, steam pelleting conditions, and calculated (estimated) metabolizable energy levels or other factors such as broiler strain cross, brooding temperatures, season of the year, and so on. Metabolizable energy is multifactorial including energy derived from carbohydrates, proteins, and lipids combined, which is part of the problem. How to estimate ME uplift raises another question, and in this report caloric conversion (ME kcal/lb body weight) by the negative control group and FCR or Adj. FCR of the other groups has been used, taking calculated ME of diets also as the basis for energy intake.

TME Uplift by Enzymes Using Rooster Assay. Adult White Leghorn rooster true metabolizable energy (TME) assays were conducted using 2 corn-soy and animal-vegetable fat diets with different calculated ME values (1,379 or 1,424 kcal ME/lb). These diets had calculated crude protein and fat values of 19.8 and 19.3%, and 6.30 and

4.34%, respectively. The enzyme product was added at 0 or 0.05% (0 or 1 lb/ton). Eight roosters were trained to consume 40-50 g of feed within 2 hours. The 4 dietary treatments were administered to the roosters on 4 sequential days, excreta collected, and bomb calorimetry conducted. Results are shown in Table 12.

Table 12. Calculated metabolizable energy (ME) on an as fed basis of 2 diets and results of adult rooster true metabolizable energy (TME) assays with or without the enzyme product (0 or 0.05%) on a 100% dry matter basis (Bailey, 2005)

	Moisture, %	Calculated ME, kcal/kg (kcal/lb)	Actual TME, kcal/kg DM ± s.d. (kcal/lb)
Lower energy control	9.39	3,040 (1,379)	3,220 ± 84 (1,461 ± 38) [b]
Lower energy + enzymes	9.28	3,040 (1,379)	3,283 ± 99 (1,489 ± 99) [ab]
Higher energy control	10.28	3,140 (1,424)	3,290 ± 108 (1,492 ± 49) [ab]
Higher energy + enzymes	10.32	3,140 (1,424)	3,373 ± 157 (1,530 ± 71) [a]

[a–b]Means for TME having a different letter superscript differ ($P \leq 0.05$).

In the lower energy diet, the enzyme product gave a +28 kcal TME/lb DM uplift (+26 kcal TME/lb as fed basis). In the higher energy diet, the enzyme product yielded a +38 kcal/lb DM improvement. (+34 kcal/lb as fed basis). The ME/TME ratios for the lower energy control and lower energy + enzymes diets were 94.41 and 92.60%, respectively. The ME/TME ratios for the higher energy control and higher energy + enzymes diets were 95.44 and 93.09%, respectively.

The main difference in these basal diets was crude fat levels of 4.34 versus 6.30% so the enzymes may be interacting with dietary fat to improve utilization. For each 1% crude fat, the enzyme product appeared to derive 5.99 kcal extra TME/lb lower energy diet and 5.40 extra kcal TME/lb higher energy diet on an as fed basis. The TME values can be converted to ME values for (immature) broiler chickens or laying hens, and TME values are perhaps directly most relevant to White Leghorn hens as their male counterparts (adult roosters) were used to conduct assays in this trial.

The National Research Council's Nutrient Requirements of Poultry (1994) lists corn as having MEn of 1,520 kcal/lb and TMEn of 1,574 kcal/lb. The MEn value is 96.54% of the TMEn value, and these values usually differ because MEn is obtained with broiler chickens whereas TMEn is usually determined with adult White Leghorn roosters. Soybean meal solvent extracted without hulls has MEn of 1,107 kcal/lb and TMEn of 1,127 kcal/lb, so MEn/TMEn is 98.19%. In Trial 8, the lower energy diet had 66.69% corn and 27.79% soybean meal whereas the higher energy diet had 62.87% corn and 29.53% soybean meal. This helps explain why the determined TME values were higher than the calculated ME values.

Conclusion. Three relatively new commercial enzyme products, REAP® 4M for mash feeds, REAP® TS for steam pelleted feeds, and Liquid REAP for post-pelleting feed application, providing the same enzyme activities per ton of feed (β-glucanase, 200,000 units and protease 5,000 HUT), were evaluated in 7 broiler litter pen trials and in an adult White Leghorn rooster assay from 2003 to 2007.

Significant improvements in body weight and feed conversion ratio or mortality-adjusted feed conversion ratio were typical, generally without affecting mortality. A meta-analysis involving 17 paired comparisons (negative control versus enzyme diets with 0.0412% average enzyme product inclusion rate) from 6 trials revealed improvements of +0.180 lb in body weight and -0.100 in feed conversion ratio or adjusted feed conversion ratio at an average of 41.9 days without affecting mortality.

Based on results from 6 trials, it was found that at an average inclusion rate of 0.040% enzyme product, a +68.1 kcal/lb feed apparent uplift in metabolizable energy occurred (range +34 to +115 kcal ME/lb feed). Linear regression of ME uplift on enzyme product level was nonsignificant indicating variability. The enzyme product significantly reduced starch content of fresh excreta samples from broilers. In a 49-day trial, the 0.025% level of enzyme product was found to be equivalent to 26.83 lb poultry fat/ton (1.34%), and the 0.25% level of enzyme product was equivalent to 41.83 lb poultry fat/ton (2.09%) in the 1.00% poultry fat basal diets. Assuming 3,850 kcal ME/lb for poultry fat, 1.34% would provide +52 kcal ME/lb feed and 2.09% would contribute +80 kcal ME/lb feed. (average +66 kcal ME/lb at 0.0375% enzyme inclusion rate; equivalent to 1.72% added poultry fat).

Using improvements in body weight, chilled carcass weight, and breast meat weight from a broiler grower-finisher trial, the enzyme blend product was estimated to contribute an apparent +1.37% extra crude protein to the diet due to better protein utilization.

Adult White Leghorn rooster true metabolizable energy (TME) assays of diets with 2 metabolizable energy levels, with or without enzymes, revealed apparent TME uplifts of +26 and +38 kcal/lb feed on an as fed basis due to enzyme supplementation (0.05%). Broiler calculated kcal ME/lb values for the 2 diets were 92.6% to 95.4% of the actual kcal TME/lb feed results. The TME results are also directly applicable to laying hens because the TME testing was done with their male counterparts (adult roosters).

The enzyme blend product REAP® is recommended for improving metabolizable energy (carbohydrate, protein, and fat) utilization in broiler chicken feeds. On average the apparent uplift in metabolizable energy was +68 kcal/lb across all levels of inclusion of enzymes tested (0.0125 to 0.05%, mean 0.040%). This was approximately equal to 1.72% added poultry fat (+66 kcal ME/lb feed uplift on average, based on responses to 0.025 and 0.05% enzymes).. The enzyme blend was found to be equivalent to an extra 1.37% dietary crude protein during the 21-42 day grower-finisher period for improving broiler body weight, chilled carcass weight, and breast meat weight.

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